NADP-linked enzyme. Moreover calculations based on oxygen uptake during flight (see Tucker, 1968) of the pigeon show that the rate of the cycle is several times higher than the activity of the NAD-linked ICDH (activities having been adjusted for the temperature difference). Thus it is suggested either that vertebrate NAD-linked ICDH is less stable than that from insect flight muscle or that the NADP-linked enzyme must play a role in the oxidation of substrates by the tricarboxylic acid cycle in these muscles.

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The Effects of Calcium Ions and Adenosine Diphosphate on the Activity of Nicotinamide-Adenine Dinucleotide-Linked Isocitrate Dehydrogenase of Muscle

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The NAD-linked ICDH* is considered to be a regulatory enzyme for the tricarboxylic acid cycle in muscle. Previous work has shown that ADP is a specific activator of the enzyme, and therefore changes in the ADP concentration may regulate the rate of the cycle (Chen & Plaut, 1963; Goebell & Klingenberg, 1964). It is known that Ca^{2+} is important in the link between nervous stimulation and mechanical activity in muscle (see Weber, 1966), and that low concentrations of Ca^{2+} can activate the mitochondrial enzyme glycerol 1-phosphate dehydrogenase (Hansford & Chappell, 1967). Therefore effects of Ca^{2+} on NAD-linked ICDH of various muscles were investigated.

The activity of ICDH was measured by following the reduction of NAD^+ by crude extracts or mitochondrial preparations from insect flight muscles (e.g. *Lethocerus cordofanus*, *Locusta migratoria*, *Sarcophaga barbata*) and rat heart muscle. It was found that the activating effect of ADP on ICDH was dependent on the Ca^{2+} concentration, which was controlled by the use of Ca^{2+} -ethanedioxybis(ethyl-

amine)tetra-acetate buffers. At a minimal Ca^{2+} concentration (1mM) the enzyme was maximally active in the absence of any added ADP, so that addition of ADP up to 2mM had no further effect on the enzyme activity. However, at 10 μM - Ca^{2+} and in the absence of added ADP the activity was extremely low, but was increased to a maximal rate by addition of ADP. Raising the Ca^{2+} concentration from 10 μM to 1mM had no further effect on the activity in the absence of added ADP and did not change the response of the enzyme to increasing ADP concentrations. It is suggested that the effects of Ca^{2+} and ADP on ICDH are independent.

These effects of Ca^{2+} demand that any theory of the control of ICDH activity should include this ion. There is some evidence to suggest that mitochondria may play a complementary role to the sarcoplasmic reticulum in the control of the intracellular distribution of Ca^{2+} ; thus mitochondria might absorb Ca^{2+} during relaxation of muscle, and release it into the sarcoplasm during contraction (Weber, 1966; Patriarca & Carafoli, 1969). As ICDH is located in the mitochondrial matrix, a high intramitochondrial Ca^{2+} concentration would inhibit the enzyme whereas a low concentration would activate it. Thus it is suggested that the effects of Ca^{2+} and ADP, which are independent at the enzymic level, provide a concerted regulatory mechanism that ensures that the activity of ICDH is extremely low during rest, yet is maximal when the muscle is mechanically active.

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Control of Respiratory and Fermentative Balance in Yeast

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It has been previously suggested that the fermentative carbon source controls the respiratory capability of the yeast *Saccharomyces cerevisiae* (Tustanoff & Bartley, 1964). To further investigate this hypothesis this species of yeast was grown

* Abbreviation: ICDH, isocitrate dehydrogenase.

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aerobically on several fermentable sugars. Cells grown on glucose, galactose and maltose manifested a decreased respiratory capacity per cell mass (Q_{O_2}) while the original carbon source was being metabolized; however, once fermentation ceased (as monitored by ethanol production) the Q_{O_2} increased markedly. This decrease in respiration (i.e. repression) is proportional to the rate of fermentation (glucose > maltose > galactose). If these respiratory data are plotted semi-logarithmically as total respiration (μmol of O_2 /min per ml of culture) against time the three sets of data fall progressively on the same straight line. This unexpected result suggests that some respiration-limiting substance is being synthesized at an identical rate that is independent of the carbon source in all three cultures.

To exploit this latter observation yeast cells were grown aerobically on 2.7% galactose medium in the presence and absence of D(-)-chloramphenicol, an antibiotic that inhibits mitochondrial protein synthesis (Clark-Walker & Linnane, 1967). Chloramphenicol had no effect on the synthesis of hexokinase, alcohol dehydrogenase, malate dehydrogenase or isocitrate dehydrogenases while galactose was still present in the medium. The effect of this inhibitor on succinate dehydrogenase and pyruvate decarboxylase activities was gradual and cumulative, resulting in a 25% inhibition. In contrast, the synthesis of cytochrome *c* reductase and cytochrome *c* oxidase was immediately affected and curtailed by chloramphenicol. Strikingly the synthesis of these last two enzymes was exponential, insensitive to change of carbon source and paralleled by the total respiratory activity. The fermentative and tricarboxylic acid-cycle enzymes on the other hand were sensitive to change of metabolic carbon source (i.e. from galactose to ethanol) and demonstrated parabolic kinetics during synthesis. Genetic evidence from petite mutation (Sherman, 1965) suggests that mitochondrial DNA is responsible in part for the activities of cytochrome *c* oxidase and cytochrome *c* reductase, and the chloramphenicol data (Clark-Walker & Linnane, 1967) showed that these enzymes are initially synthesized in part at least in the mitochondrion. Thus the 'limiting metabolite' referred to above is most probably a protein that may be assembled from incomplete cytoplasmic protein by mitochondrial DNA, and the latter experiment indicates that the synthesis of this substance is not affected by the external environment of the cell and is insensitive to nuclear cytoplasmic control (cf. Ball & Tustanoff, 1968).

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Subcellular Fractionation of Anaerobically Grown *Saccharomyces carlsbergensis* by Zonal Centrifugation

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Sphaeroplasts were prepared under strictly anaerobic conditions from yeast cells grown anaerobically with 10% (w/v) glucose in the presence of Tween 80 and ergosterol. Cycloheximide (0.1mg/ml) and chloramphenicol (1mg/ml) were added to prevent any possibility of respiratory adaptation during the disruption and centrifugation procedures, which were identical with those described in the following communication (Cartledge, Howells & Lloyd, 1970).

The activities of cytochrome *c* oxidase and succinate-cytochrome *c* oxidoreductase in whole homogenates were not detectable (<0.5nmol/min per mg of protein), nor was that of catalase (<0.2nmol/min per mg of protein).

Equilibrium-density centrifugation in the B XIV zonal rotor yielded two populations of organelles with median buoyant densities of (A) 1.147 and (B) 1.21g/ml, which were detected by their absorption at 520nm. Both these populations contained antimycin A-sensitive NADH-cytochrome *c* oxidoreductase activity; both populations also showed NADPH-cytochrome *c* oxidoreductase. The specific activity of adenosine triphosphatase in population A was ten times that in B; oligomycin-sensitivity was evident in population A and not in population B.

The distribution of NADH- and NADPH-cytochrome *c* oxidoreductase and NADPH-ferricyanide oxidoreductase showed a complex distribution in the region of the gradient ρ 1.10, which indicated that, although a portion of all three activities is non-sedimentable, some of the activities are associated with slowly sedimenting particles. Acid phosphatase showed a sharp distribution in the non-sedimentable region and a broader sedimentable zone at median buoyant density 1.14.

Rate-zonal centrifugation confirmed that NADH- and NADPH-cytochrome *c* oxidoreductase and NADH- and NADPH-ferricyanide oxidoreductase as well as adenosine triphosphatase are associated with rapidly sedimenting particles. The percentage total enzyme units present in this population were as follows: protein, 4.5%; NADH-cytochrome *c* oxidoreductase, 25%; NADPH-cytochrome *c* oxidoreduc-